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Research Article

Effectiveness of "bahu-baho" *Lantana camara L.* leaf extract as alternative larvicide on household mosquito *Culex pipiens* larvae

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ABSTRACT

The study was conducted to determine the effectiveness of Bahu-Baho *Lantana camara L* leaf extract as alternate larvicide against mosquito *Culex pipiens* larvae. It utilized experimental design which has four treatment levels with three replications each. The set-up was the control, 25%, 50% and 75% concentration of bahu-baho leaves extract. Using ANOVA the result showed that there was no significant difference on the mortality of mosquito larvae with regards to the different treatment levels in a given time interval. With the toxicity level of Bahu-baho extract and the given time interval, it further entailed that the shorter the period of time of exposure of mosquito larvae to the concentration of bahu-baho leaves extracts, the higher the level of concentration it required to exterminate the given percentage of mosquito larvae. On the other hand, the longer the time of exposure, the lower the level of concentration needed to achieve the desired lethal dose effect on the larvae.

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INTRODUCTION

Mosquitoes are common insects that breed quickly and are regarded as pests because of the risk of harm they pose to humans. Nt only will these mosquitoes ruin the backyard, but their nuisance biting and virus transmission may also infuriate nearby residents. Aside from the annoyance they may cause, these pests must be controlled because they are potential carriers of diseases, particularly vector-borne diseases like malaria, dengue fever, leishmaniasis, and yellow fever (WHO, 2014).

In the year 2022, the Department of Health (DOH)'s Disease Surveillance reported more than 220,000 dengue cases in the Philippines from January 1 to December 17, 2022 (Philippine Star, Jan 17, 2023). This is 182 percent higher than the 78,223 noted cases during the same period in 2021. DOH's Epidemiology Bureau also showed that dengue-related deaths increased in 2022 with 722, or a case fatality rate (CFR) of 0.3 percent. During the same period in 2021, 282 deaths or a CFR of 0.4 percent were recorded. Central Visayas got the highest number of dengue deaths with 103 individuals, this was followed by Central Visayas, 99, and Western Visayas, 85. These figures reflect that dengue is one of the fastest-spreading viral diseases and children are the most vulnerable. This outbreak necessitates urgent and collaborative actions from all sectors of the government in order to contain it and not cause too much harm to the public. Thus, controlling the widespread of mosquitoes becomes vital and requires effective public health intervention. Mosquitoes can typically lay 100 to 200 eggs at a time, and they produce eggs every third night. The female mosquito will die after producing three sets of eggs, totaling 300 to 600 offspring. The eggs hatch and the mosquitos continue their life cycle, mating, reproducing, and dying. They suck blood and depend on standing water to reproduce. Female mosquitoes must feed on blood to lay eggs. They feed by sticking their mouthparts into the skin of an animal and suck blood rapidly. More often, they carry viruses that can be transmitted to a person while they are feeding (Mosquito-Borne Diseases, as cited by Nasiru, et al., 2014).

One of the ways to halt the proliferation of these mosquitoes is to control them by killing their larvae for which most people used chemical insecticides in doing it. Though these chemical insecticides provide great benefits, they can potentially cause adverse health effects on both the environment and humans. The effects may affect to all but children have a great risk for their physiological and development due to their toxic content (Menon, 2005). According to Sipe, et al, (2011), mosquito control is a very important and effective public health intervention that has reduced the prevalence of Ross River virus in Australia. At present, the easiest and fastest way to control them is the use of commercial and synthetic chemicals available in the market. These chemicals are potentially destructive and expensive. More so, synthetic chemicals are among the sources of pollutants. They have also a negative impact, like toxic residues in food, water, air, and soil, resurgence and resistance of insect pests, and effects on non-target organisms (Ahmad, et al. 2013).
These are the reasons that the study of larvicidal activity of Lantana camara Linn (Verbenaceae) against the larvae of common species of mosquitoes found within the Philippines was investigated aiming to develop natural pesticides derived from plants as means of destroying pests that are not only affordable for all walks of life with but also with fewer adverse effects on one's health.

Objectives

The study aimed to determine the effectiveness of Bahu-baho (Lantana camara L.) leaf extract as alternative larvicide agent on household mosquito (Culex pipiens) larvae.
Specifically, it sought to answer the following specific objectives.

1. To determine the level of effectiveness of Bahu-baho (Lantana camara L.) leaf extracts on the mortality rate of mosquitos (Culex pipiens) Larvae across different levels of concentration at a given period of time;

2. To determine the significant difference on the level of effectiveness of Bahu-baho (Lantana camara L.) leaf extracts on the mortality rate of mosquitos (Culex pipiens) Larvae across different levels of concentration at a given period of time;
3. To determine the lethal dose of Bahu-baho (Lantana camara L.) leaf extract in exterminating mosquito Culex pipiens larvae.

Hypothesis

Ho1. There is no significant difference on the level of effectiveness of Bahu-baho (Lantana camara L.) leaf extract as larvicide at different levels of concentration.

METHODS

The study utilized a post-test-only control group experimental design. This kind of research method is the "only method of research that can truly test hypotheses concerning cause-and-effect relationships. Hence, considered as the appropriate research design to determine the effectiveness of Bahu-baho (Lantana camara L.) leaf extract as alternative larvicide agent on household mosquito (Culex pipiens) larvae. It represents the most valid approach to the solution of educational problems, both practical and theoretical, and to the advancement of education as a science" (Gay, L. R, 1992).

Subject of the Study

The household mosquito, Culex pipien larvae were breed and screened on the basis of the mosquito's morphological characteristic, the length and shape of the antennae. The plant (LANTANA camara L.) were gathered in one area of Sagay.

Materials

The materials used during the experiment were mosquito larvae, pale, bahu-baho leaves, fish bath tub, water jug, netted spoon, mortar and pestle, blender, cheese cloth, glass bottle, tap water, funnel, graduated cylinder, distilled water, petri dish, netted spoon, glass container, clock, pad paper, pen, camera, lab gown with headdress. After the experiment calculator for solving, detergent and brush for cleaning, graduated cylinder for measuring extract, tap water for cleaning, lint free cloth for filtering and disposable plastic bags for garbage were used.

Experimental Set-up

Plant extraction

The Bahu-baho leaves were collected and were separated from its stem. One thousand grams of leaves were soaked overnight. On the next day, the soaked leaves were drained, pounded, macerated, and filtered to get the extract for treatment.

Larvae preparation

Three (3) pail of water approximately 30 Liters were placed in an area where there were plenty of mosquitoes to lay eggs. This served as the breeding station of the mosquitoes. The breeding place is monitored daily for the development of eggs to the point where the eggs were hatched. The hatched eggs were monitored daily and were harvested after seven (7) days. The larvae were then transferred to a water jug to let them stay for one (1) day before the application of treatment.
Larvicidal testing

The concentration were calibrated as: solute/solution x 100%. And were varied from twenty five percent (25%), fifty percent (50%), and (75%) extract by volume respectively at variation of time of 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, and 24 hours. There were four treatment levels used. Treatment 1 is the control group with zero percent (0%) concentration of the extract. Treatment 2 used twenty-five percent (25%) concentration having 12.5 mL extract/37.5 mL water. Treatment 3 used fifty percent (50%) concentration having 25 mL extract/25 mL water. Treatment 4 used seventy-five percent (75%) concentration having 37.5 mL extract/12.5 mL water. The prepared Bahu-baho extract at varying concentrations were place in glass containers with three (3) replications for each treatment level, a total of twelve (12) glass containers containing mosquito larvae were prepared as replication. These twelve (12) replications in all treatments were observed at an interval of four (4) hours, eight (8) hours, twelve (12) hours, sixteen (16) hours (20) hours and twenty four (24) hours. Thirty (30) larvae were a on each glass container and observed by the researcher for the duration of twenty-four (24) hours. The number of dead larvae in each glass container were counted and recorded by the researcher.

Table 1. Experimental setup

Concentration of	% Concentration of	Replication																Total
		1					2					3						
		4 HR	8 HR	12 HR	16 HR	20 HR	24 HR	4 HR	8 HR	12 HR	16 HR	20 HR	24 HR	4 HR	8 HR	12 HR	16 HR	20 HR	24 HR	
T_1	control	10 mosquito larvae					10 mosquito larvae						10 mosquito larvae							30
T_2	12.5 mL extract /37.5 mL water	10 mosquito larvae					10 mosquito larvae						10 mosquito larvae							30
T_3	25 mL extract /25 mL	10 mosquito larvae					10 mosquito larvae						10 mosquito larvae							30

	water				
T_4	37.5 ml extract /12.5 mL water	10 mosquito larvae	10 mosquito larvae	10 mosquito larvae	30
					120
		TOTAL MOSQUITO LARVAE			

RESULTS AND DISCUSSION

Table 2. Mean result on the mortality rate of mosquito larvae at different level of treatment in a given period of time

Treatment	Rep	4hrs	8hrs	12hrs	16hrs	20hrs	24hrs	total
A	1	0	0	0	0	0	0	
A	2	0	0	0	0	0	0	
A	3	0	0	0	0	0	0	
Mean		0	0	0	0	0	0	
B	1	2	3	3	4	6	9	
B	2	4	6	6	6	7	8	
B	3	7	7	7	7	7	8	
Total		4.33	5.33	4.33	5.66	6.66	8.33	
C	1	5	7	7	8	9	10	
C	2	2	9	9	9	9	9	
C	3	5	6	6	6	7	9	
Mean		4	7.33	7.33	7.67	8.33	9.33	
D	1	7	8	8	8	9	10	
D	2	4	6	6	6	8	10	
D	3	6	8	8	8	8	9	

Mean	5.67	8	8	7.33	8.33	9.66

Among the four treatments at varying concentration in a given period of time, the highest recorded mean after (4) four hours were on treatment 3 having 75% concentration with average mean of 5.67. After 8 hours to 12 hours, the same result of treatment 3 having 50% and treatment 4 having 75% concentration with the average mean of 7.33 were recorded. After the sixteen (16) hours, treatment 3 with 50% concentration got the highest recorded mean of 7.67. On the other hand, after twenty (20) hours, results show that treatment 3 having 50% concentration and treatment 4 with 75% concentration got the same mean of 8.33. Finally, after twenty-four hours observation the highest recorded mean was found on treatment 4 having 75% concentration with As a whole, considering the different treatment levels after the number of hour's observation, the results revealed that there were no significant difference found on the mortality rate of mosquito larvae applied with different treatment levels of bahu-baho leaves extracts in a given period of time. This implies that regardless of the treatment levels of Bahu-baho leaves extract applied, it foster similar effect in exterminating mosquitos Culex pipiens larvae. The high rate of mortality may be due to a component in the essential oil known as terpenoids in the methanol extracts of the leaves of Lantana camara (Sathish & Maneemegalai 2008).

Table 3. Mean and difference analysis results on the mortality of mosquito Culex pipiens larvae as applied with the different treatment levels of bahu- baho leaf extracts in a given period of time

Treatment	Means (4hrs. after)	Means 8(hrs. after)	Means (12hrs. after)	Means (16hrs. after)	Means (20hrs. after)	Means (24hrs. after)
0%	0.00	0.00	0.00	0.00	0.00	0.00
25%	4.33	5.33	5.33	5.67	6.67	8.33
50%	4.00	7.33	7.33	7.67	8.33	9.33
75%	5.67	7.33	7.33	7.33	8.33	9.67
p-value	0.579	0.296	0.296	0.256	0.073	0.068
Decision	Fail to reject	Fail to reject	Fail to reject	Fail to reject	Fail to reject	Fail to reject
conclusion	Not Sig	Not Sig	Not Sig	Not Sig	Not Sig	Not Sig

Not Sig. – If p-value is greater than 0.05

Table 3 reflect significant difference on the level of effectiveness of Bahu-baho (LANTANA camara L.) leaf extracts on the mortality rate of mosquitos (Culex pipiens) larvae across different levels of concentration at a given period of time. As a whole, analysis of the results showed that the p-value in all treatment levels were greater than 0.05 level of significance. Hence, four treatment levels with respect to the specified period of time that the mosquito Culex pipiens larvae were exposed to varying concentration of Bahu-baho extract were found to have no significant difference. This implies that regardless of the different treatment levels of bahu-baho extract the level of effectiveness on the mortality mosquito Culex pipiens larvae across different level of concentration at a given period of time were evidently observed in the table.

Table 4. Significant difference analysis results on the mortality of household mosquitos as applied with the different treatment levels of bahu- baho leaf extracts in a given period of time

Treatment	Means (4 hrs. after)	Means (8 hrs. after)	Means (12 hrs. after)	Means (16 hrs. after)	Means (20 hrs. after)	Means (24 hrs. after)
0%	0.00	0.00	0.00	0.00	0.00	0.00
25%	4.33	5.33	5.33	5.67	6.67	8.33
50%	4.00	7.33	7.33	7.67	8.33	9.33
75%	5.67	7.33	7.33	7.33	8.33	9.67
p-value	0.579	0.296	0.296	0.256	0.073	0.068
Decision	Fail to reject	Fail to reject	Fail to reject	Fail to reject	Fail to reject	Fail to reject
Conclusion	Not Sig	Not Sig	Not Sig	Not Sig	Not Sig	Not Sig

Not Sig. – If p-value is greater than 0.05

Probit Analysis Results on the Mortality of household mosquitos as Applied with the Different Treatment Levels of Bahu- baho leaf extracts after 4hrs. The result showed that 62.77688 % extract/50 ml water was the required concentration of bahu-baho leaves extract to achieved lethal dose 50% of the mortality of mosquito larvae after 4 hours' time observation. Meanwhile, for lethal dose 90%, recorded concentration of 8657.324 % extract/50 ml water was needed. Thus, implied that there was no significant difference found on the mortality of household mosquito applied with different levels of bahu-baho leaves extracts with the specified time given. This implies that the Bahu-baho leaf extract should increase its level of concentration to foster significance on the mortality of mosquito larvae.

Table 4.a. Probit Analysis Results on the Mortality of household mosquitos as Applied with the Different Treatment Levels of Bahu- baho leaf extracts after 4hrs

Lethal Dose 50 (LD50)	62.77688 % extract/50 ml water
Lethal Dose 90 (LD90)	8657.324 % extract/50 ml water

Chi square value of 0.5510 with P value of 0.4543

Table 4.b. Probit analysis results on the mortality of household mosquitos as applied with the different treatment levels of bahu- baho leaf extracts after 8hrs.

Lethal Dose 50 (LD50)	19.6965 % extract/50 ml water
Lethal Dose 90 (LD90)	221.9537 % extract/50 ml water

Chi square value of 0.1300 with P value of 0.7184

Probit Analysis Results on the Mortality of household mosquitos as Applied with the Different Treatment Levels of Bahu- baho leaf extracts after 12hrs. Table 4.b showed that with the use of probit analysis, results revealed to have no significant difference found on the mortality of household

mosquito as applied with different treatment levels of bahu- baho leaves extract after 12 hours. This implies that the bahu-baho leaf extract foster the same effectiveness across the period of time.

Table 4.c. Probit analysis results on the mortality of household mosquitos as applied with the different treatment levels of bahu- baho leaf extracts after 12hrs

Lethal Dose 50 (LD50)	19.6965 % extract/50 ml water
Lethal Dose 90 (LD90)	221.9537 % extract/50 ml water

Chi square value of 0.1300 with P value of 0.7184

Probit Analysis Results on the Mortality of household mosquitos as Applied with the Different Treatment Levels of Bahu- baho leaf extracts after 16hrs. For table 4.d, results revealed that after 16 hours of observation, there was no significant difference recorded on the mortality rate of mosquito larvae in relation to the different treatment levels of bahu-baho extract applied. This implies that the toxicity of of bahu-baho leaf extract foster the same effect across the different levels of concentration across the period of time. The result above revealed that, 19.6965 % extract/50 ml water was the desired concentration of the treatment to achieved lethal dose 50% of the mortality of mosquito larvae after 8 hours' time duration. Meanwhile, for lethal dose 90%, recorded concentration of 221.9537 % extract/50 ml water was needed. No significant difference recorded with this observation. This implies that mosquito larvae were able to develop resistance to the bahu-baho extract.

Table 4.d. Probit analysis results on the mortality of household mosquitos as applied with the different treatment levels of bahu- baho leaf extracts after 16hrs

Lethal Dose 50 (LD50)	15.4109 % extract/50 ml water
Lethal Dose 90 (LD90)	242.2478 % extract/50 ml water

Chi square value of 0.2215 with P value of 0.6379

Probit Analysis Results on the Mortality of household mosquitos as Applied with the Different Treatment Levels of Bahu- baho leaf extracts after 20hrs. Probit Analysis results showed that with the different treatment levels of bahu-baho leaves extract applied on mosquito larvae after 20 hours, found to have no significance difference. This implies that the lethal dose of bahu-baho leaf extract after 20 hours foster the same effect across the period of time due to emergence of larvicidal resistance on the level of toxicity across different levels of concentration.

Table 4.e. Probit analysis results on the mortality of household mosquitos as applied with the different treatment levels of bahu- baho leaf extracts after 20hrs.

Lethal Dose 50 (LD50)	10.3861 % extract/50 ml water
Lethal Dose 90 (LD90)	115.0153 % extract/50 ml water

Chi square value of 0.0676 with P value of 0.7948

Probit Analysis Results on the Mortality of household mosquitos as Applied with the Different Treatment Levels of Bahu- baho leaf extracts after 24hrs. The lethal dose of Bahu-baho leaves extract in exterminating mosquito Culex pipiens larvae as reflected on table 8.f revealed that there was no significant difference found on the mortality of mosquito larvae after 24 hours observation applied with different treatment levels of bahu-baho leaves extract. This implied that with toxicity level of Bahu-baho extract and the given time interval, it further entails that the shorter the period of time of exposure of mosquito larvae to the concentration of bahu-baho leaves extracts, the higher the level of concentration it requires to exterminate the given percentage of mosquito larvae. On the other hand, the longer the time of exposure, the lower the level of concentration needed to achieve the desired lethal dose effect to the larvae. Thus, the lethal dose determination of bahu-baho leaves extracts depend on the given time duration of exposure which was found to be indirectly proportional to the level of concentration applied (Zhu et. al., 2008) studies have reported the effectiveness of plant extracts or essential oils, especially (Lantana camara L.) which is a weed known for its insecticidal properties, against mosquito larvae.

Table 4.f. Probit Analysis Results on the Mortality of household mosquitos as Applied with the Different Treatment Levels of Bahu- baho leaf extracts after 24hrs

Lethal Dose 50 (LD50)	7.2871 % extract/50 ml water
Lethal Dose 90 (LD90)	37.4212 % extract/50 ml water

Chi square value of 0.0000 with P value of 0.9945

The results showed that there were no significant difference on the effect on the four treatments and its treatment levels on the mortality rate of mosquito larvae after theapplication. It revealed that T1 which is the control group has no recorded dead larvae while on the T4 which has 75% concentration has the highest recorded mean of 9.67 with a p value of 0.068 which is greater than 0.05 level of significance. However, with toxicity level of Bahu-baho extract and the given time interval, it further entails that the shorter the period of time of exposure of mosquito larvae to the concentration of bahu-baho leaves extracts, the higher the level of concentration it requires to exterminate the given percentage of mosquito larvae. On the other hand, the longer the time of exposure, the lower the level of concentration needed to achieve the desired lethal dose effect to the larvae. Thus, the lethal dose determination of bahu-baho leaves extracts depend on the given time duration of exposure which was found to be indirectly proportional to the level of concentration applied (Zhu et al., 2008) studies have reported the effectiveness of plant extracts or essential oils, especially (Lantana camara L.) which is a weed known for its insecticidal properties, against mosquito larvae.

CONCLUSION

Based on the results treatment four which has the highest concentration of 75% (37.5 mL extract/12.5 mL water) had the higher recorded mortality rate of mosquito larvae after twenty four (24) hours' observation with the mean of 9.67. This means that the Bahu-baho leaf extract has potential effect use as larvicide with higher concentration level. The shorter the period of time of exposure of mosquito larvae to the concentration of bahu-baho leaves extracts, the higher the level of concentration it requires to exterminate the given percentage of mosquito larvae. On the other hand, the longer the time of exposure, the lower the level of concentration needed to achieve the desired lethal dose effect to the larvae. Thus, the lethal dose determination of bahu-baho leaves extracts depend on the given time duration of exposure which was found to be indirectly proportional to the level of concentration applied. There was no significant difference found on the level of effectiveness of Bahu-baho (LANTANA camara L.) leaves extract as larvicide at different levels of concentration.

RECOMMENDATION

Based on the foregoing findings, the following were recommended:

1. The extract from the leaves of the Bahu-baho (Lantana camara L) plant with 75% concentration of 37.5 mL extract per 12.5 mL water has potential effect on exterminating Culex pipiens larvae at the first 4 hours observation, however it should increase its level of concentration to obtain its significance on the mortality of mosquito larvae.
2. Bahu-baho plants grown anywhere in rural areas and the extract from its leaf can be used as an alternative larvicide at a dose of LD50 62.77688 %extract/50 mL water and LD90 with 8657.324% extract/mL water which is environmental friendly and less costly.
3. For best result, higher concentration of Bahu-baho leaf extract should be applied in mosquitoes breeding area and some places where there are prevalent population of mosquitos.
4. It is also recommended that same study considering the different species of mosquito larvae shall be conducted to determine its level of effectiveness.
5. Based on the probit analysis results on the lethal dose effect of bahu-baho leaves extract in relation to the mortality of mosquito larvae, higher concentration level of the treatment was needed to effectively exterminate the mosquito larvae in a given short period of time.

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